CCLVI. THE OXIDATION PRODUCTS OF THE UNSATURATED ACIDS OF LINSEED OIL

By LESLIE CHARLES ALFRED NUNN AND IDA SMEDLEY-MACLEAN

Biochemical Department, Lister Institute

GREEN & HILDITCH [1935] investigated the effects of various oxidizing agents on the unsaturated acids of linseed oil and found that the best yields of hydroxy-acids were obtained by the action of alkaline permanganate, though even with this the yields were low; from linoleic acid, 40 % tetrahydroxystearic acid was obtained whilst the yield of hexahydroxy-acids from linolenic acid was only 15 to 18 % of that theoretically possible. These authors also investigated the oxidation of pure α-linoleic acid and isolated from it 65 % of the theoretical yield of tetrahydroxy-acid when they worked under the conditions described by Hazura [1888] and 38 % when the conditions given by Lapworth & Mottram [1925] were used. β-Linoleic acid oxidized under Hazura's conditions furnished only 5 % of the tetrahydroxy-compound. They concluded that the α-form of linoleic acid is the one normally present in natural oils and that the β-compound is a complex mixture of transposition products, more readily degraded by oxidation than is the original α-acid.

In 1935 we published a preliminary communication on the oxidation of the acids of linseed oil by means of alkaline permanganate: the proportions of the various hydroxy-acids formed were determined and the presence of lactonic acids established amongst the degradation products.

The methods available for the analysis of linseed oil or of any oil containing more than two unsaturated acids are still far from satisfactory. The solid bromination products obtained on bromination of linoleic and linolenic acids represent only a small proportion of the total bromides, and bromine determinations have therefore to be carried out on the various fractions of the liquid bromides. Only if the amount of one of the three unsaturated acids present is known can the proportions of the other two be calculated from the i.v. Kaufmann's "thiocyanogen number" [Kaufmann & Keller, 1929], which has been used to determine the amounts of linoleic and linolenic acids, depends on arbitrary assumptions and in the hands of different observers gives very variable results [1] [cf. Gay, 1932]. The conversion of pure oleic acid into dihydroxystearic acid is, however, almost theoretical. Lapworth & Mottram [1925] found that, working with 1 % KMnO$_4$ in very dilute solutions, a 96 % yield of the dihydroxy-acid was obtained. They found, however, that when as much as 12 to 13 % linoleic acid was present with the oleic acid, the total precipitate containing both the di- and tetra-hydroxy-acids corresponded only with 96 % of the amount of hydroxy-acid theoretically obtainable. They add: "Judging from the results of experiments which the authors have made on the production of hydroxyketostearic acid, the yield of dihydroxystearic acid may fall off very rapidly if the proportion of acids more highly unsaturated than oleic acid exceeds a certain maximum." It is not, however, stated what conditions were used in these experiments on the forma-

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[1] Griffiths & Hilditch [1934] found the method quite satisfactory if sufficient care were taken to exclude all traces of moisture.

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tion of the hydroxyketo-acid, but they almost certainly must have differed from those in which only the hydroxy-acids were produced.

In our experience using Rollett's [1909] conditions of oxidation, the conversion of oleic acid into dihydroxystearic acid is remarkably constant even in the presence of considerable quantities of the higher unsaturated acids.

15 g. acids obtained by the saponification of linseed oil were dissolved in 18 ml. 33 % KOH solution and 3 l. water added. The solution was cooled to 0° and 2 l. 1 % $KMnO_4$ solution previously cooled to 0° added during a period of 30 min., the mixture being constantly stirred and cooled with ice so that the temperature never rose above 5°; SO_2 was then immediately passed in until the MnO_2 was dissolved, and the solution acidified with dilute H_2SO_4. The precipitate, which at once separated, was filtered off, dried and extracted for 24 hr. with boiling light petroleum (B.P. 60–80°). The residue was then thoroughly extracted with boiling water to remove tetra- and hexa-hydroxystearic acids, dried and weighed. In three similar experiments it corresponded to 96 % of the dihydroxy-acid theoretically obtainable from the oleic acid present.

Two examples of crude oleic acid analysed in this way gave the following results.

(a) A commercial specimen, purified once by the Pb salt-alcohol method, I.V. 87·8. Two determinations were made, 5 g. acid being used for each. After allowing for the 0·95 g. saturated acid, the I.V. corresponded to 65 % oleic and 16·1 % linoleic acid. The percentage of oleic acid calculated from a 96 % yield of the dihydroxy-compound was 64·5.

Weight in g. of	(a)	(b)	Mean
Crude hydroxy-acid ppt.	5·14	5·24	5·19
Petrol-soluble saturated acids	0·88	1·01	0·95
Dihydroxy-acid	3·46	3·48	3·47
Tetrahydroxy-acid	0·34	0·32	0·33
Water-soluble residue	0·51	0·49	0·50

(b) A fraction of the unsaturated acids from butter which had been separated by distillation of the methyl esters and the unsaturated acids purified by the Pb salt-alcohol and Li salt-acetone processes gave 75·6 % oleic acid calculated from the I.V. and 75·4 % calculated from the dihydroxy-acid. Calculated from the I.V. 9·0 % linoleic acid was present.

The composition of the Calcutta linseed oil, I.V. 180, used for the oxidation experiments was investigated by the above method. After determining the proportions of saturated and oleic acids present, the amounts of linoleic and linolenic acids were calculated from the I.V. of the original mixture of acids.

	%
Saturated acids	10·90
Oleic acid	12·96
Linoleic acid	26·00
Linolenic acid	43·60
Unsaponifiable matter	1·20

Two determinations of the saturated acids, using the Pb salt-alcohol method of separation, were also made and gave 9·2 and 8·9 % saturated acids. When oxidizing larger quantities of material the best results were obtained if a preliminary separation of part of the saturated acid was first effected by applying the Pb salt-alcohol process. The unsaturated fraction was then worked up. Oxidation of 225 g. of unsaturated acids in 30 g. portions yielded hexahydroxy-acids corresponding to 39·9–51·6 % of the linolenic acid originally present and tetrahydroxy-acids corresponding to 27·6–32·3 % of the linoleic acid.

Table I. *Showing yields of oxidation products obtained from the oxidation of 225 g. linseed oil unsaturated acid fraction*

	(1) g.	(2) g.	M.P.
(1) Saturated acids	12·5	9·5	—
(2) Dihydroxystearic acid	36·0	38·2	132°
(3) Tetrahydroxystearic acid, soluble in boiling, insoluble in cold water	19·0	22·2	156–165°
(4) Hexahydroxystearic acids:			
(1) Linusic acid (obtained from aqueous extract after separation of the tetrahydroxy-acid)	36·0	49·0	180–195°
(2) *iso*Linusic acid (obtained as oily ppt. on further concentrating aqueous filtrate from ppt. of hydroxy-acids. Purified by ether extraction)	18·8	20·8	173–175°
(5) Lactonic acid. $C_{12}H_{18}O_4$. Dark oil extracted by ether from *iso*linusic fraction	—	16·7	Oil
(6) Viscid yellow oil obtained by further concentrating aqueous filtrate. Contains lactonic acid $C_{12}H_{20}O_5$, small quantities of azelaic acid and other acids	—	75·2	Oil
(7) Soluble substances readily reducing $KMnO_4$, remaining in aqueous filtrate	—	—	—
(8) Volatile acids. Formic and *n*-hexoic present and probably propionic	—	—	—

It was estimated that fractions (1) to (6) contained approximately 80% of the carbon of the acids oxidized.

The nature of the oil extracted by ether from the *iso*linusic acid fraction

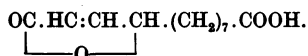This oil was not examined in our preliminary investigation.

After removal of the ether, a yellow limpid oil remained which gradually changed on standing in the desiccator to a dark brown viscous substance, sparingly soluble in ether, some form of polymerization possibly taking place. The oil was extracted with benzene and the portion soluble both in ether and in benzene examined.

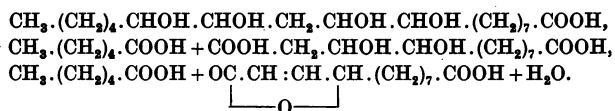|	Analysis of fractions			Equivalent	
	% C	% H	I.V.	Neutralization	Saponification
(1) Benzene- and ether-insoluble	65·60	8·60	18·8	—	176·9
(2) Benzene-insoluble, ether-soluble	62·20	7·59	19·6	262·0	168·9
(3) Benzene- and ether-soluble	63·47	7·92	27·6	236·6	159·4
Theory for $C_{12}H_{18}O_4$	63·70	8·10	112·0	226·0	122·0

The clear yellow solution on standing deposited a brown viscous oil insoluble in benzene, so that polymerization possibly again took place in the benzene solution.

The difference between the equivalents obtained by direct titration and by saponification suggested the presence of a large proportion of lactonic acids. The analysis of the benzene-soluble oil agreed with the formula $C_{12}H_{18}O_4$; the difference between the neutralization and saponification values indicated the presence of the lactone of a hydroxy-dibasic acid. The actual determination of the end-point of the saponification value was difficult owing to the dark brown colour of the solution after boiling with alcoholic KOH. The discrepancy was, however, too big to be entirely explained in this way, and either the lactone ring was incompletely opened after 1 hr. boiling with alcoholic KOH or else some monobasic acid was present as impurity. The low I.V. obtained is typical of the

behaviour of the $\alpha\beta$-unsaturated acids. These data indicate that the constitution of the ether- and benzene-soluble oil is as follows:

$$OC.HC\!:\!CH.CH.(CH_2)_7.COOH.$$
$$\underline{\qquad O \qquad}$$

This would be derived from tetrahydroxystearic acid by the following reactions:

$$CH_3.(CH_2)_4.CHOH.CHOH.CH_2.CHOH.CHOH.(CH_2)_7.COOH,$$
$$CH_3.(CH_2)_4.COOH + COOH.CH_2.CHOH.CHOH.(CH_2)_7.COOH,$$
$$CH_3.(CH_2)_4.COOH + OC.CH\!:\!CH.CH.(CH_2)_7.COOH + H_2O.$$
$$\underline{\qquad O \qquad}$$

Evidence was subsequently obtained that the same lactonic acid was produced when pure linoleic acid was similarly oxidized. The very small proportion of azelaic acid isolated from these oxidation products seems to be explained by the tendency of the oxidized molecules to split between the 12th and 13th rather than between the 9th and 10th carbon atoms, leaving the comparatively stable lactonic acids.

Fraction obtained by further concentration of the aqueous solution after separation of the isolinusic fraction

After separating the *isolinusic* fraction, the solution was concentrated to a small bulk and cooled; a yellow viscid oil separated, from which, by extraction with limited quantities of hot water, 5 g. azelaic acid crystals were isolated.

Benzene-soluble fractions of the residual brown oil were analysed: C, 58·1, 58·8 %; H, 8·07, 8·18 %. The lactonic acid $C_{12}H_{20}O_5$ requires C, 59·01; H, 8·19 %. The percentage of carbon was considerably lower than that in the unsaturated C_{12} lactonic acid accompanying the *isolinusic* acid. Lactonic acids were, however, present in the oil since the equivalents determined by direct titration and by saponification were respectively 184·8–206·7 and 119–127. No more solid azelaic acid separated.

A further purification was effected by fractionation of the Na salts from solution in absolute alcohol. The dry salts obtained by neutralization with $N/2$ NaOH in the cold were extracted with absolute alcohol in which more than 50 % of the whole amount dissolved.

The alcohol-soluble Na salt contained C, 53·20; H, 7·26; Na 7·99 %. $C_{12}H_{19}O_5$ Na requires C, 52·16; H, 6·89; Na 8·33 %. This fraction appeared, therefore, to consist mainly of the Na salt of the γ-lactone of the 3:4-dihydroxydecane-dicarboxylic (1 : 10) acid $C_{12}H_{20}O_5$ represented above as an intermediate stage in the formation of the unsaturated γ-lactonic acid $C_{12}H_{18}O_4$. The existence of this lactonic acid suggested in our preliminary paper [1935] is therefore confirmed.

The alcohol-insoluble Na salt was recrystallized from dilute alcohol, the presence of a trace of water very greatly increasing its solubility. The percentage of Na varied from 10·1 to 15·83 in different fractions.

Originally we endeavoured to separate the constituents of this oily fraction by fractionation of the Zn salts. A small proportion of a Zn salt was obtained, the analysis and the equivalent of which agreed closely with those required by the formula $C_{12}H_{22}O_6Zn$ [1935]. In repeating this work, we again obtained a Zn salt which was, as before, less soluble in hot water than in cold and which, therefore, separated on warming its solution. We found, however, that on re-dissolving the salt in cold water and warming the solution, only a slight amount

was precipitated and it did not behave as a homogeneous substance.. On acidifying the solution, an oily acid separated which gradually deposited a small amount of crystals of azelaic acid. Possibly an unstable double salt of Zn azelaate with the Zn salt of a higher acid is formed. From the evidence now available it seems probable that this fraction contained the lactonic acid of the β-hydroxy-acid corresponding with the unsaturated lactonic acid described above. We cannot confirm the isolation of the Zn salt of the dihydroxydibasic acid: in the light of further experience, it seems probable that the free $\beta\gamma$-dihydroxydibasic acid would be at once converted into the corresponding lactonic acid.

The oxidation of linoleic acid

In the first experiment, 90 g. linoleic acid were obtained from the solid tetrabromide isolated from the bromination of the unsaturated acids of maize oil [Nicolet & Cox, 1922]. The I.V. was 150 and it contained about 9 % saturated acids. The procedure already described for the oxidation of the linseed oil acids was followed for the oxidation of the linoleic acid. After the precipitate containing the tetrahydroxystearic acid and the saturated acids had been separated, the solution was further concentrated until the approximate concentration at which the *iso*linusic fraction separated from the oxidation product of the linseed oil acids had been reached. An oil then separated, completely soluble in ether and amounting to 11·4 % of the original weight of linoleic acid taken. Further concentration resulted in the precipitation of 12·2 g. of an oil from which a small quantity of azelaic acid (1·2 %) was crystallized.

90 % of the oil corresponding in its water-solubility with the "*iso*linusic fraction" was extracted with benzene and possessed the following properties:

| | % C | % H | I.V. | Equivalents | |
				Neutral-ization	Saponification
Benzene-soluble oil	65·88	9·53	2·05	272	184
Corresponding fraction from linseed oil acids	63·47	7·92	27·6	236·6	159·4

The benzene-soluble oil contained, therefore, some compound with a higher percentage of carbon than that of the C_{12} lactonic acid.

Determination of hydroxyl group

The lactone was esterified and the hydroxyl value of the ethyl ester estimated by the Zerewitinoff method. 0·2841 g. ethyl ester gave 12·28 ml. CH_4 at N.T.P. This would correspond with the presence in the oil of 55 % of the dibasic acid $C_{12}H_{20}O_5$ formed by opening the lactone ring.

Reduction

(a) *By sodium amalgam.* After treating with sodium amalgam in faintly acid solution for 12 hr., micro-determinations of the C and H contents of the oil showed that the substance was unaltered. No readily reducible ethylene linkage was present in the molecule.

(b) *By platinum oxide and hydrogen.* 0·45 g. absorbed 25 ml. hydrogen (N.T.P.). The equiv. wt. of the lactonic acid is 226 and 226 g. oil had absorbed 12·55 l., i.e. 0·56 mol. hydrogen. This determination together with the low I.V. (2·03) suggested the presence of 55 % of a substance containing an $\alpha\beta$-ethylene linkage; the close agreement of this figure with that obtained in the determination of the

hydroxyl group suggested that 55% of the $\alpha\beta$-unsaturated lactonic acid was present together with 45% of some saturated non-hydroxylated substance containing a higher percentage of carbon. The product gave no precipitate with dinitrophenylhydrazine so that the presence of a keto-group could be excluded. Since our starting material was linoleic acid and the product was saturated, dioxidostearic acid presented itself as a possible constituent, as it is saturated and contains no hydroxyl group.

The carbon and hydrogen percentages and the neutralization and saponication equivalents of a mixture of 45% dioxidostearic acid with 55% of the lactone of hydroxy(3)-decenic-(1:2)-dicarboxylic acid (1 : 10) would be:

	% C	% H	Equivalent	
			By neutral-ization	By saponifica-tion
Theory	66·07	9·04	264·7	202·5
Benzene-soluble oil	65·88	9·53	—	—
	65·55	9·57	272	184

These results appeared sufficiently near to justify the conclusion that the oil consisted of a mixture of these two substances. We endeavoured to confirm this by reducing the mixture with HI and isolating the completely reduced acids.

Reduction with HI in boiling acetic anhydride [Crowder & Anderson, 1932]. 0·7 g. oil was boiled for 3 hr. with HI (sp. gr. 1·7) and red phosphorus in acetic anhydride solution. From the reaction product treated according to the directions given, stearic acid, M.P. 68°, was isolated by extraction with light petroleum and its identity confirmed by mixed M.P. From the petroleum-insoluble fraction a very small quantity of glistening white crystals soluble in hot water was obtained: these melted at 122–124°. *n*-Decanedicarboxylic acid was synthesized from ω-bromo-undecanic acid, for a specimen of which we are indebted to Dr J. C. Smith; the dicarboxylic acid melted at 125–126°, and admixture with the specimen obtained by reduction of the lactonic acid gave no depression. The crystals gave no evidence of lactonic properties. The presence of a C_{18} compound and of a derivative of the C_{12} dicarboxylic acid was thus confirmed. 0·7 g. dioxidostearic acid similarly reduced yielded 0·22 g. stearic acid.

The lactonic acid $C_{12}H_{18}O_4$ obtained as an oxidation product of the mixed linseed oil acids has, therefore, been identified amongst the oxidation products of pure linoleic acid. The presence of dioxidostearic acid appears not to have been previously noticed among the products of oxidation of linoleic acid by alkaline permanganate, although it is formed when oxidation is carried out by perbenzoic acid [Green & Hilditch, 1935].

Proportion of linoleic acid recovered as tetrahydroxy-acid
under Rollett's conditions of oxidation

In the first experiment carried out 90 g. linoleic acid (I.V. 150) (from which the saturated acid had not been completely removed) were oxidized. From the I.V. the proportion of pure linoleic acid was 83·1%; the weight of pure acid oxidized was therefore 74·8 g.; 50 g. tetrahydroxy-acid were isolated—a yield of 53·8%. In a second experiment, 30 g. linoleic acid, I.V. 166·7, were oxidized and 18·1 g. tetrahydroxy-acid recovered, a yield of 52·8%. The percentages of linoleic acid degraded were therefore 46·2 and 47·2. Green & Hilditch [1935] found a 65% yield of the tetrahydroxy-acid using Hazura's [1888] conditions, and 38%

when the conditions described by Lapworth & Mottram [1925] were employed. As we only isolated 27·6–32·3 % tetrahydroxy-acid from the linseed oil mixed acids, we confirm Green and Hilditch's results that when the mixed unsaturated acids are oxidized, a far larger proportion of the tetrahydroxy-acid is broken down than when pure linoleic acid is oxidized. In view of the finding of these authors, that the oxidation products of β-linoleic acid are almost completely degraded to lower carbon compounds, possibly the effect of the presence of linolenic acid is to increase the isomerization of linoleic acid from the α- to the β-form.

Oxidation of linolenic acid

Two experiments were carried out using linolenic acid prepared from the solid hexabromide obtained in the bromination of the linseed oil acids. The I.V. was 258 corresponding to 94·1 % of pure linolenic acid. 15 g. quantities were used for the oxidations: the yields of linusic acids were respectively 10·7 and 8·3 % and of *iso*linusic 14·7 and 14·0, making a total percentage yield of hexahydroxy-acids of 25·4 and 22·3 %. Green and Hilditch give 18 % as their yield. When the pure linolenic acid was oxidized about 75% was degraded to compounds containing smaller numbers of carbon atoms, but when the mixed linseed oils were oxidized the proportion of linolenic acid broken down to lower carbon compounds was only 55–64 %. The linoleic acid in the mixture appears, therefore, to exercise a protective action on the linolenic acid.

CONCLUSIONS

1. The products of oxidation of the mixed acids of linseed oil by means of alkaline permanganate under Rollett's conditions have been investigated.

2. The proportion of the various oxidation products has been determined.

3. The 96 % yield of dihydroxy-acid obtained from oleic acid under these conditions was very little affected by the proportions of linoleic and linolenic acid present.

4. The estimation of oleic acid in a mixture of unsaturated acids is most conveniently made as dihydroxystearic acid.

5. The yields of tetrahydroxy-acids from the linoleic acid present in the mixed linseed oil acids were only 28–32 %: yields of 52–54 % were obtained when pure linoleic acid was similarly oxidized.

6. The yields of hexahydroxy-acids calculated on the linolenic acid present in the mixed acids were 40–52 %. When pure linolenic acid was similarly oxidized, only 22–25 % hexahydroxy-acid was separated. Linoleic acid is therefore more readily degraded in the presence of linolenic acid and exercises a protective action on the oxidation of the linolenic acid.

7. The greater part of the degradation products consists of γ-lactonic acids containing 12 carbon atoms. The presence of the acid $C_{12}H_{18}O_4$ has been established and its constitution determined as the γ-lactone of hydroxy-(3)-decenic (1:2)-dicarboxylic acid (1:10). The same acid has been shown to occur amongst the degradation products of pure linoleic acid.

8. Evidence of the existence of the γ-lactone of dihydroxy-(3:4)-decanedicarboxylic acid has been obtained.

9. The amount of azelaic acid formed on oxidation under the given conditions is very small (1·2 %).

10. Fission takes place more readily between the 12:13 carbon atoms carrying the hydroxyl groups than between those in the 9:10 positions.

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